Adipose tissue concentrations of persistent organic pollutants and total cancer risk in an adult cohort from Southern Spain: preliminary data from year 9 of the follow-up

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Abstract

There is an increasing trend in the incidence of cancer worldwide, and it has been accepted that environmental factors account for an important proportion of the global burden. The present paper reports preliminary findings on the influence of the historical exposure to a group of persistent organic pollutants on total cancer risk, at year 9 in the follow-up of a cohort from Southern Spain.

A cohort of 368 participants (median age 51 yrs) was recruited in 2003. Their historical exposure was estimated by analyzing residues of persistent organic pollutants in adipose tissue. Estimation of cancer incidence was based on data from a population-based cancer registry. Statistical analyses were performed using multivariable Cox-regression models.

In males, PCB 153 concentrations were positively associated with total cancer risk, with an adjusted hazard ratio (95% confidence interval) of 1.20 (1.01-1.41) for an increment of 100 ng/g lipid.

Our preliminary findings suggest a potential relationship between the historical exposure to persistent organic pollutants and the risk of cancer in men. However, these results should be interpreted with caution and require verification during the future follow-up of this cohort.

Keywords

Persistent organic pollutants; adipose tissue; cancer; prospective study; hazard ratio; follow-up.

1. Introduction

There is an increasing trend worldwide in the incidence of cancer, and predictions for 2030 include annual rates of 27 million incident cases of cancer and 17 million cancer-related deaths annually (Ferlay J et al., 2010;International Agency for Research on Cancer, 2008). This trend cannot be solely explained by the improvement in diagnostics or by the ageing of populations. In addition, wide disparities in the incidence of the most frequent types of cancer have been reported across the five continents, possibly due to complex interactions between genetic susceptibility and modifiable risk factors (Kamangar et al., 2006).

It has traditionally been accepted that environmental factors account for a large proportion of cancers, reaching up to 80-90% of the global burden of the disease (International Agency for Research on Cancer, 2008). Specifically, an estimated 6% of total cancer deaths is attributable to occupational or environmental exposure to carcinogenic agents, which appears to be a small percentage in comparison to other known causes, such as tobacco consumption, but translates into a large number of individuals in the general population (American Cancer Society, 2014). Furthermore, little is known about the health outcomes derived from exposure to complex mixtures of environmental pollutants that can interact with each other and with internal elements of the organism (American Cancer Society, 2014;Kortenkamp, 2006). In this regard, the European Code against Cancer emphasized the need to apply strict regulations aimed at preventing any exposure to known cancer-causing substances in order to decrease the cancer risk (Boyle et al., 1995).

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs), i.e., highly lipophilic chemicals that are very resistant to degradation and tend to accumulate and biomagnify in food chains, resulting in the considerable exposure of living organisms (UNEP, 2003). OCPs have been used in many agricultural activities and as vector control (Porta et al., 2002), while PCBs have been

employed as dielectric and heat exchange fluids, among other commercial applications (WHO, 2000). Although the use of most OCPs and PCBs has been banned or severely restricted in most countries, these chemicals are still detected in virtually all human populations and environmental matrices, and diet (especially fatty food) has been reported to be the main route for human exposure (Brauner et al., 2012a).

There is increasing scientific evidence that exposure to low levels of POPs, such as those occurring in the general population, is related to an increased risk of several adverse health effects, including some of the most prevalent types of cancer, e.g. hepatocellular carcinoma (Zhao et al., 2011), breast cancer (Salehi et al., 2008), cancer of the head and neck (Govett et al., 2011), non-Hodgkin lymphoma (Engel et al., 2007), prostate cancer (Xu et al., 2010), testicular germ cell tumors (McGlynn et al., 2008), or pancreatic cancer (Hardell et al., 2007). Additionally, long-term occupational exposure to PCBs has been associated with elevated melanoma mortality (Ruder et al., 2013). However, there have been conflicting reports on the relationship between exposure to low levels of POPs, such as those occurring in the general population, and cancer risk, with some studies reporting positive associations but many others finding no evidence to support a causal association (Cassidy et al., 2005;Charlier et al., 2003;Cohn et al., 2010;Gatto et al., 2007;Govett et al., 2011;Hardell et al., 2006;Hoyer et al., 2001;Laden et al., 2001;Lopez-Carrillo et al., 2002;Lopez-Cervantes et al., 2004;Mozzachio et al., 2008;Pavuk et al., 2004;Recio-Vega et al., 2011;Snedeker, 2001;Soto and Sonnenschein, 2010;Svensson et al., 1995;Ward et al., 2000;Wolff et al., 2000;Xu et al., 2010).

The present paper reports preliminary results on the influence risk of the historical exposure to a group of organochlorine pesticides and polychlorinated biphenyls on total cancer, at year 9 during the follow-up of an adult cohort from Southern Spain.

2. Material and Methods

2.1 Study cohort

The present research is part of a hospital-based study that aimed to characterize the exposure to POPs of an adult cohort from Southern Spain and assess potential health outcomes. The study design, recruitment, and methods have been extensively described elsewhere (Arrebola et al., 2013a;Arrebola et al., 2010;Arrebola et al., 2009). In brief, Granada province covers an area of 12 635 km^2 in Southern Spain. Study subjects were recruited in two public hospitals, San Cecilio University Hospital in the city of Granada (240 000 inhabitants) and Santa Ana Hospital in the town of Motril (50 000 inhabitants).

Study participants were recruited between July 2003 and June 2004 among patients undergoing non-cancer-related surgery (47% inguinal hernia or abdominal surgery, 17% gall bladder surgery, 12% varicose vein surgery, and 24% other surgery). Inclusion criteria were: age over 16 years, absence of cancer, not undergoing hormone therapy, and residence in one of the study areas for ≥10 years. All subjects signed their informed consent to participate in the study, which was approved by the ethics committees of both hospitals. Out of 409 subjects who were contacted, 387 agreed to participate. A total of 19 subjects were excluded because of a previous diagnosis of cancer, leaving a final cohort of 368 participants. All of the participants were users of the public health system. No statistically significant differences in sex distribution or age were found between participants and non-participants (data not shown in tables). The characteristics of the study population are summarized in Table 1.

2.2 Exposure assessment

2.2.1 Sampling and chemical analyses

During surgery, samples of 5-10 g adipose tissue were collected, immediately coded, and stored at -80 °C until chemical analysis. The sample extraction and purification was

previously described by Rivas et al. (2001). In brief, 200 mg of adipose tissue were extracted using n-hexane, and the solution was then purified through 2 g alumina in a glass column. All extracts were stored in glass tubes at -80º C.

POPs were quantified by high-resolution gas chromatography with a mass spectrometry detector in tandem mode, using a system Saturn 2000 ion trap (Varian, Walnut Creek, CA). For the analysis, we used a 2 m × 0.25 mm silica capillary column (Bellefonte, PA) coupled with a 30 m × 0.25 mm analytical column (Factor FOUR VF-5MS, Varian Inc., Walnut Creek, CA). For all measured POPs, the limit of detection were set at 0.01 μg/L. Chromatographic concentrations below the limit of detection were assigned a random value between 0 and the limit of detection. In adipose tissue, residues of p,p´-dichlorodiphenyldichloroethylene (p,p´-DDE, the main metabolite of the pesticide dichlorodiphenyltrichloroethane [DDT]), hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), and PCB congeners -138, -153 and -180 were quantified. Recoveries of the POPs from adipose tissue were studied to assess the extraction efficiency of the method used, and ranged from 90–98%.

Lipid content in adipose tissue samples was quantified gravimetrically as reported by Rivas et al. (2001), including a homogenization step of 100 mg adipose tissue with 5 mL of chloroform:methanol:hydrochloric acid (20:10:0.1) and acidification with hydrochloric acid 0.1N before collecting and weighing the organic phase.

In adipose tissue samples, lipid-basis concentrations were calculated by dividing the crude adipose tissue concentrations by the total lipid content and were expressed in nanograms per gram lipid (ng/g lipid).

2.2.2 Total effective xenoestrogen burden (TEXB)

In order to calculate the overall estrogenicity of the adipose tissue extracts, samples were tested in the E-Screen bioassay, which measures the proliferative effect of xenoestrogens on MCF-7 breast cancer cells by comparing cell yield between cultures of MCF-7 cells treated with estradiol and those treated with different concentrations of xenobiotics or extracts (Soto et al., 1992). Each adipose tissue extract was resuspended in 5 mL Dulbecco's modified Eagle's medium without phenol red, supplemented with 10% charcoal dextran-treated human serum, and was then tested in the E-Screen bioassay for estrogenicity at dilutions of 1:1, 1:5, and 1:10, using a slight modification of the originally described technique. Each sample was assayed in triplicate with a negative (vehicle) and positive (estradiol) control in each plate. The proliferative effect of the adipose tissue extract was referred to the maximal effect obtained with estradiol, transformed into estradiol equivalents (Eeq) units by reading from a dose–response curve, and expressed in Eeq units per gram of lipid.

In the present study, we quantified the estrogenicity of the whole adipose tissue extract, whereas previous studies of the TEXB in biological samples have measured the estrogenicity of two fractions of each extract, collected using a preparative normal-phase high performance liquid chromatography protocol: the alpha-fraction, which includes non-polar xenoestrogens (e.g. OCPs and PCBs); and the beta-fraction, which contains more polar xenoestrogens, sex steroids, and pharmaceutical estrogens (Fernandez et al., 2004). For the present study, no high performance liquid chromatography fractionation was performed and the overall estrogenicity of the whole extract was designated as TEXB-extract. We previously reported that the TEXB-extract yields information about the overall estrogenicity to which humans are exposed and may be useful to assess its potential contribution to health outcomes (Arrebola et al., 2012a).

2.3 Outcome assessment

New cancer cases were ascertained using data from the population-based Granada Cancer Registry (http://cancergranada.org/es/index.cfm), which began its activity in 1985 and provides information for cancer surveillance and control in the region of Granada. It covers a population of approximately 900,000 inhabitants and serves as a basis for cancer epidemiological research. Cancer was defined as the diagnosis of any malignant neoplasm and classified according the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10, codes C00-C97, except non-melanoma skin cancer) (WHO, 2013). Follow-up time started at the date of recruitment and continued until the diagnosis of cancer or the patient's death. If the patient did not experience either of these events, this preliminary follow-up period ended on 31 December 2012, although the cohort remains under study.

2.4 Covariates

Data on socio-demographic characteristics, lifestyle, and health status were gathered in face-to-face interviews conducted by trained personnel during the hospital stay. Questionnaires and research procedures were standardized and validated in a pilot study with 50 subjects.

Body mass index (BMI) was expressed as weight/height squared (Kg/m^2). A subject was considered a smoker (past or present) with any level of daily tobacco consumption ($\geq$1 cig/day). Subjects were classified into six occupational categories, following Goldthorpe's proposal (Regidor, 2001).

2.5 Statistical methods

The descriptive analysis included the calculation of means, standard deviations, medians, and percentiles for the interval variables, and percentages for the categorical variables. $\sum$PCBs and $\sum$POPs were computed as the sum of individual adipose tissue concentrations of the three

PCB congeners (-138, -153 and -180) and all six POPs (p,p´-DDE, HCB, β-HCH, and PCBs -138, -153, and -180), respectively. In the bivariate analyses, variables were compared using the Mann-Whitney U-test and Fisher's exact test as appropriate.

The disease-free survival and the time-to-events were estimated from the date of recruitment, date of diagnosis, and date of the end of the follow-up. Data on subjects who died before the observation of a study outcome were censored; therefore, only their disease-free time was considered in the analyses.

The statistical analyses were performed using Cox-regression models, estimating hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). We selected a set of potential confounders based on the literature (Table 1) and constructed multivariable models that were sequentially adjusted for those associated ($p<0.200$) with cancer risk or POP concentrations in the bivariate analyses, i.e., age, BMI, and smoking habit. A final model was constructed adjusted for all three covariates. POP concentrations were always treated as continuous variables.

Data were stored and processed using R statistical computing environment v3.0 (http://www.r-project.org/). The significance level was set at $p \leq 0.05$ and all tests were two-tailed.

3. Results

Out of the 368 adults enrolled in this study, 22 were diagnosed with cancer (Table 2), i.e., a cumulative incidence of 6%. Median follow-up time, including censored and non-censored data, was 75.1 months both in men and women. Incidence density in our population was 83.93×10^{-5} person-years. Table 2 shows the classification of cancer incident cases according to tumor site. Females showed a cumulative incidence of 5%, an incidence density of 69.77 x

10^{-5} person-years, and the most common tumor localizations were rectum, skin (melanoma), and breast. Females diagnosed with cancer were older than those who were not (median age of 63 vs. 49 yrs, respectively, p=0.021) and had a larger number of children (median number of 3.3 vs. 2.3, respectively, p=0.051), but there was no significant difference in BMI (median value of 27.9 vs. 25.9 kg/m^2, respectively, p=0.166) (data not shown in tables). The cancer incidence was 7% in males, predominantly in lung and prostate, and the incidence density was 97.00 x 10^{-5} person-years. Males diagnosed with cancer were older than those who were not (median age of 58 *vs.* 49 yrs, respectively, p=0.060), and they had a higher BMI (median value of 30 *vs.* 27 kg/m^2, respectively, p=0.004) (data not shown in tables).

Median POP concentrations ranged from 16.1 ng/g lipid (β-HCH) to 231.4 ng/g lipid (PCB 153) in females, and from 7.3 ng/g lipid (β-HCH) to 212.3 (PCB 153) in males (Table 1). Frequencies of detection in females and males were, respectively, 93% and 90% (PCB 153), 92% and 89% (PCB 180), 88% and 85% (PCB 138), 93% and 87% (HCB), and 87% and 81 % (β-HCH). All study subjects showed detectable concentrations of *p,p´*-DDE. All POPs showed statistically significant positive correlations between them, with Spearman coefficients ranging from 0.6 to 0.9 (data not shown in tables).

Table 3 shows the Cox-regression analyses of the relationship of POP concentrations with total cancer risk, both unadjusted and sequentially adjusted for the potential confounders. HRs were calculated for an increment of 100 ng/g lipid in the POP concentrations to assist the results interpretation. In general, the inclusion of covariates in the model slightly reduced the HR magnitude. In women, no significant associations were found between POP concentrations and the risk of cancer. In men, however, concentrations of PCB 153 (statistically significant) and PCB 138 (borderline significant) were positively associated with

cancer risk at all levels of adjustment, with HRs of 1.19-1.26 (PCB 153) and 1.46-1.78 (PCB 138). The main results of the analyses are also summarized in Figure 1.

No statistically significant associations were observed between TEXB-extract level (overall estrogenicity of the adipose tissue sample) and total cancer risk in either females or males.

4. Discussion

In the present preliminary study, total cancer risk in men was associated with concentrations of PCB 138 (statistically significant) and PCB 153 (borderline significant) in an apparently monotonic manner. In men, we observed a rise in cancer risk of 20% (95% CI: 1-41%) for an increase of 100 ng/g lip in adipose tissue PCB 153 concentrations. The fact that these associations were not found in women might be due to the lower number of female cases or because of sex-related physiological differences. In this regard, Bräuner et al. (2012b) prospectively studied the influence of adipose tissue POP concentrations on the development of non-Hodgkin lymphoma and found stronger associations in men than in women; the authors proposed potential sex differences in the metabolism and elimination rate of POPs as a possible explanation. However, the sex-related differences in the HRs found in the present study are of interest, and deserve to be confirmed in the further follow-up of our cohort.

Many OCPs and PCBs have been proven to interact with estrogen and/or androgen receptors (Sonnenschein and Soto, 1998;Soto et al., 1998) and, therefore, epidemiological efforts have generally been focused on hormone-dependent tumors, e.g. breast and prostate (Xu et al., 2010). However, other researchers have suggested potential non-endocrine-related mechanisms of action of POPs, including an increase in reactive oxygen species and reactive nitrogen species through the induction of cytochrome P450 or mitochondrial alterations (Karami-Mohajeri and Abdollahi, 2011) and a disruption of the epigenomic landscape in cancers (reviewed by: Knower et al., 2014). Certain POPs have been found to induce enzymes

that produce genotoxic intermediates and DNA adducts (Yanez et al., 2004) or modulate the activity of oncogenes, such as K-ras in pancreatic cancer (Gasull et al., 2010). In the present study, we grouped together all types of cancer on the grounds that these non-hormonal mechanisms might represent a common risk factor for all cancers. This heterogeneity may explain the lack of statistically significant associations between the overall estrogenicity of the adipose tissue extracts (TEXB-extract) and the total cancer risk.

Some epidemiological evidence has been published on the link between human exposure to POPs and the risk of certain types of cancer (Boada et al., 2012;Brauner et al., 2012b;Cohn et al., 2012;Cohn et al., 2007;McGlynn et al., 2008;Recio-Vega et al., 2011;Xu et al., 2010). Thus, PCBs have been described as a risk factor for cancer (Man et al., 2011), and the entire group was recently classified as carcinogenic to humans by an IARC working group, which concluded that "the carcinogenicity of PCBs cannot be solely attributed to the carcinogenicity of the dioxin-like PCBs" (Lauby-Secretan et al., 2013). The IARC classified DDT, HCB and HCH as "possibly carcinogenic to humans (Group 2B)" (International Agency for Research on Cancer, 2012), and further prospective studies are required to fully elucidate their potential carcinogenic effect.

In our study, all analyses were stratified by sex because of potential differences between males and females in tumor localization and in cancer predictors, susceptibility, and disease progression (International Agency for Research on Cancer, 2008;Pal and Hurria, 2010). It has also been reported that metabolic rates can differ between males and females, including findings of a lower cytochrome P450 metabolism of POPs in females (McTernan et al., 2002;Moser and McLachlan, 2001;Silbergeld and Flaws, 2002).

It has been reported that obesity has a relevant impact on cancer development, contributing to 6% of incident cases of cancer in the USA (Polednak, 2008). Although the multivariable

models were adjusted for BMI, it should be taken into account that the majority of the study population (66.3%) was obese/overweight (BMI>25 kg/m^2), and that most cancers in our cohort were diagnosed among these individuals (cumulative incidence of 8.3% versus 1.6% in subjects with BMI<25 kg/m^2). On the other hand, the BMI was positively correlated with POP levels in the present cohort (Arrebola et al., 2013a;Arrebola et al., 2010;Arrebola et al., 2009) and has even been shown to be an important effect modifier of the association between POPs and diabetes (Arrebola et al., 2013b). Therefore, the potential interaction between POPs and obesity in the promotion of cancer deserves to be studied in depth. In addition, given that many POPs are also suspected of acting as obesogens, i.e., capable of promoting obesity (Sharpe and Drake, 2013), the use of BMI as a covariate might produce an overadjustment of the models. Thus, we performed a re-analysis of Model 4 without adjusting for BMI and found no relevant change in the coefficients (data not shown in tables). We therefore assume that the BMI does not introduce any significant bias in the models.

It is crucial to measure the exposure before disease onset, because adipose tissue POP concentrations can be affected by the treatment and/or by physiological changes resulting from the disease (Brauner et al., 2012b;Jandacek et al., 2005). Additionally, the use of adipose tissue concentrations to estimate historical exposure minimizes possible biases related to misclassification of the exposure. Authors have described adipose tissue as the most appropriate biological matrix for the estimation of the cumulative internal exposure to POPs because it is the main internal deposit, accounting for all routes and sources of exposure (Kohlmeier and Kohlmeier, 1995;Quintana et al., 2004). Other more readily collected matrices, such as serum, have been found to be good predictors of point exposure to POPs (including the mobilization of those stored in fatty tissues) but not always of chronic exposure (Archibeque-Engle et al., 1997;Arrebola et al., 2012b).

The main limitations of the present exploratory study are the relatively small sample size and the limited cancer incidence at year 9. Therefore, our results should be interpreted with caution and need to be confirmed in the further follow-up of this cohort. Furthermore, we were not able to compare the risk among specific cancers, which include both hormone-dependent and non-hormone-dependent types, or adjust for their individual risk factors. In addition, we did not take into account co-exposures to other chemicals, such as polybrominated diphenyl ethers (PBDEs) or phthalates, which may also be involved in the development of cancer. The relationship between the human exposure to other environmental pollutants and total cancer has been assessed in previous epidemiological studies (Blair and Freeman, 2009;Mink et al., 2012). We highlight the positive Spearman correlations observed between all POPs in our study; therefore, the associations found with single chemicals may be surrogates of exposure to other more toxic agents or even to mixtures of POPs with similar physicochemical characteristics (Arrebola et al., 2013b;Brauner et al., 2012b). These correlations were expected because, although some of the studied chemicals have different origins (e.g., agriculture or industry), their similar physicochemical properties mean that they accumulate in the lipophilic fractions of food (Duarte-Davidson and Jones, 1994;Herrera et al., 1996), which is acknowledged to be the main source of exposure in non-occupationally exposed populations (Bosch de Basea et. al., 2010).

Although information on dietary habits was available for this cohort at its recruitment (Arrebola et al., 2013a;Arrebola et al., 2010;Arrebola et al., 2009), no adjustment for food intake was performed. Diet, especially of animal origin, has been acknowledged as an important risk factor for cancer (Vieira et al., 2011) but is also responsible for most of the POP exposure in the general population (Agudo et al., 2009;Bosch de et al., 2010). We therefore assume that observed effects were mainly caused by the POPs present in the diet, and that the inclusion of diet as a covariate could produce an overadjustment of the models.

Further research is warranted to elucidate the potential mechanisms of POP toxicity and to explore the relationship between POP exposure and cancer, with the aim of identifying individuals at greater risk to support the design of primary preventive programs. Given these preliminary findings, the further follow-up of this cohort offers a promising approach for verifying the associations found.

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Conflict of interest

The authors declare no conflict of interest.

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